

PATHOLOGICAL CHANGE OF THE DEVELOPMENT OF THE VESICULAR LESION IN PIGS EXPERIMENTALLY INFECTED WITH THE FOOT-AND-MOUTH DISEASE VIRUS O/JPN/2010

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Introduction

- There are a limited number of reports on pathology in pigs infected with Foot-and-mouth Disease Virus (FMDV) within Southeast Asia (SEA) topotype.
- Especially, we are interesting in how to develop vesicular lesions in FMDV infection.
- To deepen our understanding of pathogenesis of FMDV SEA topotype in pigs, we examined pathological changes in acute stage of infection in pigs experimentally infected with FMDV isolated from the 2010 epidemic in Japan via the intraoral routes.

Materials and methods

Six pigs aged four-weeks-old were inoculated orally with 10^6 TCID₅₀ of FMDV O/JPN/2010 isolate. Each three pigs infected were examined clinically and euthanized at 1 and 3 days post inoculation (dpi).

Clinical samples of sera, saliva and nasal swabs were collected.

At necropsy, tissue samples were collected from each pig, homogenated for virus isolation and RT-PCR analysis and fixed in 10% neutral buffered-formalin for histology.

Paraffin-embedded tissue sections were stained with hematoxylin and eosin and with immunohistochemistry using monoclonal anti-FMDV antibody (NIAH). TUNEL staining (Takara Bio, Tokyo, Japan) were examined in sections from vesicular lesions to confirm whether cell death by FMDV is by apoptosis or not.

Group	Dose	PID	n
1	10^6 TCID ₅₀	1	3
2	10^6 TCID ₅₀	3	3

Gross lesions in pig inoculated with FMDV O/JPN/2010

Pig No	Dose	PID	Site of vesicular lesions	
			Tongue	Foot
2	10 ⁶	1	—	+
5		1	—	—
6		1	—	—
3		3	—	+
4		3	+	+
7		3	+	—

Clinically, vesicular lesions were observed in one of three pigs at 1 dpi and in all three pigs at 3 dpi with O/JPN/2010.



Pig No.4 3 dpi, Foot,
Ruptured vesicular lesion in the interdigital
space. OS16

Pig No.4 3 dpi, Foot, Blanching areas and vesicular lesions
at the bulb of the heel and accessory digits.

Virus shedding in the saliva, nasal swab and serum (virus isolation/RT-PCR)

Pig No.	Virus strain	Samples	0 dpi	1 dpi	2 dpi	3 dpi
2	O/JPN/2010	Saliva	-/-	-/+		
		Nasal swab	-/-	-/-		
		Serum	-/-	-/+		
5	O/JPN/2010	Saliva	-/-	1.75/+		
		Nasal swab	-/-	-/+		
		Serum	-/-	-/-		
6	O/JPN/2010	Saliva	-/-	-/-		
		Nasal swab	-/-	-/+		
		Serum	-/-	-/-		
3	O/JPN/2010	Saliva	-/-	-/-	-/+	3.0/+
		Nasal swab	-/-	-/-	-/+	2.5/+
		Serum	-/-	-/-	-/-	-/-
4	O/JPN/2010	Saliva	-/-	-/-	4.25/+	5.0/+
		Nasal swab	-/-	-/-	2.5/+	3.75/+
		Serum	-/-	-/-	4.25/+	5.0/+
7	O/JPN/2010	Saliva	-/-	-/-	-/-	1.75/+
		Nasal swab	-/-	-/+	-/-	1.75/+
		Serum	-/-	-/-	-/-	-/-

Virus shedding in saliva or nasal swab was confirmed in all pigs infected with O/JPN/2010 by RT-PCR

1.75/+ : $10^{1.75} \text{TCID}_{50}$ /PCR positive
 -/+ : Virus isolation negative/PCR positive
 -/- : negative/negative

The result of virus isolation and RT-PCR analysis from tissue samples

Pig No.	2	5	6	3	4	7
Virus strain	O/JPN/2010					
dpi	1	1	1	3	3	3
Tongue	—	—/—	—/—	—/+	2.25/+	5.0/+
Tonsil	—/+	—/—	3.5/+	—/+	5.5/+	2.5/+
Soft palate	2.0/+	—/+	—/—	—/+	3.5/+	2.5/+
Oral pharynx (lower pharynx)	1.75/+	1.75/+	—/—	1.75/+	3.5/+	1.75/+
Nasal pharynx (upper pharynx)	—/—	—/+	—/—	—/—	4.0/+	2.0/+
Larynx	2.25/+	—/+	—/—	—/—	4.5/+	2.0/+
Trachea	—/—	—/—	—/—	—/—	3.25/+	1.75/+
Lung	—/—	—/+	—/—	—/—	3.0/+	1.75/+
Esophagus	—/—	—/+	—/—	—/+	5.75/+	—/+
Stomach	—/—	—/—	—/—	—/+	2.5/+	—/—
Small intestine (ileum)	—/—	—/—	—/—	—/—	2.5/+	—/—
Large intestine (rectum)	—/—	—/—	—/—	—/—	2.5/+	—/—
Heart	—/—	—/—	—/—	—/—	3.0/+	—/+
Spleen	—/—	—/—	—/—	—/—	5.0/+	—/+
Thymus	—/—	—/—	—/—	—/—	3.0/+	1.75/+
Mandibular lymph node	—/—	—/—	—/—	—/—	6.25/+	—/+
Parotid lymph node	—/—	—/—	—/—	—/—	6.0/+	—/+
Retropharyngeal lymph node	—/—	—/—	—/—	—/—	5.0/+	—/+
Cervical lymph node	—/—	—/—	—/—	—/—	7.5/+	—/+
Mandibular gland	—/—	—/—	—/—	—/—	3.0/+	2.5/+
Parotid gland	—/—	—/—	—/—	—/—	2.5/+	—/—
Skin of the nose	—/—	—/—	—/—	—/+	6.5/+	—/—
Skin of the coronet	—/—	—/—	—/—	—/+	8.5/+	4.5/+

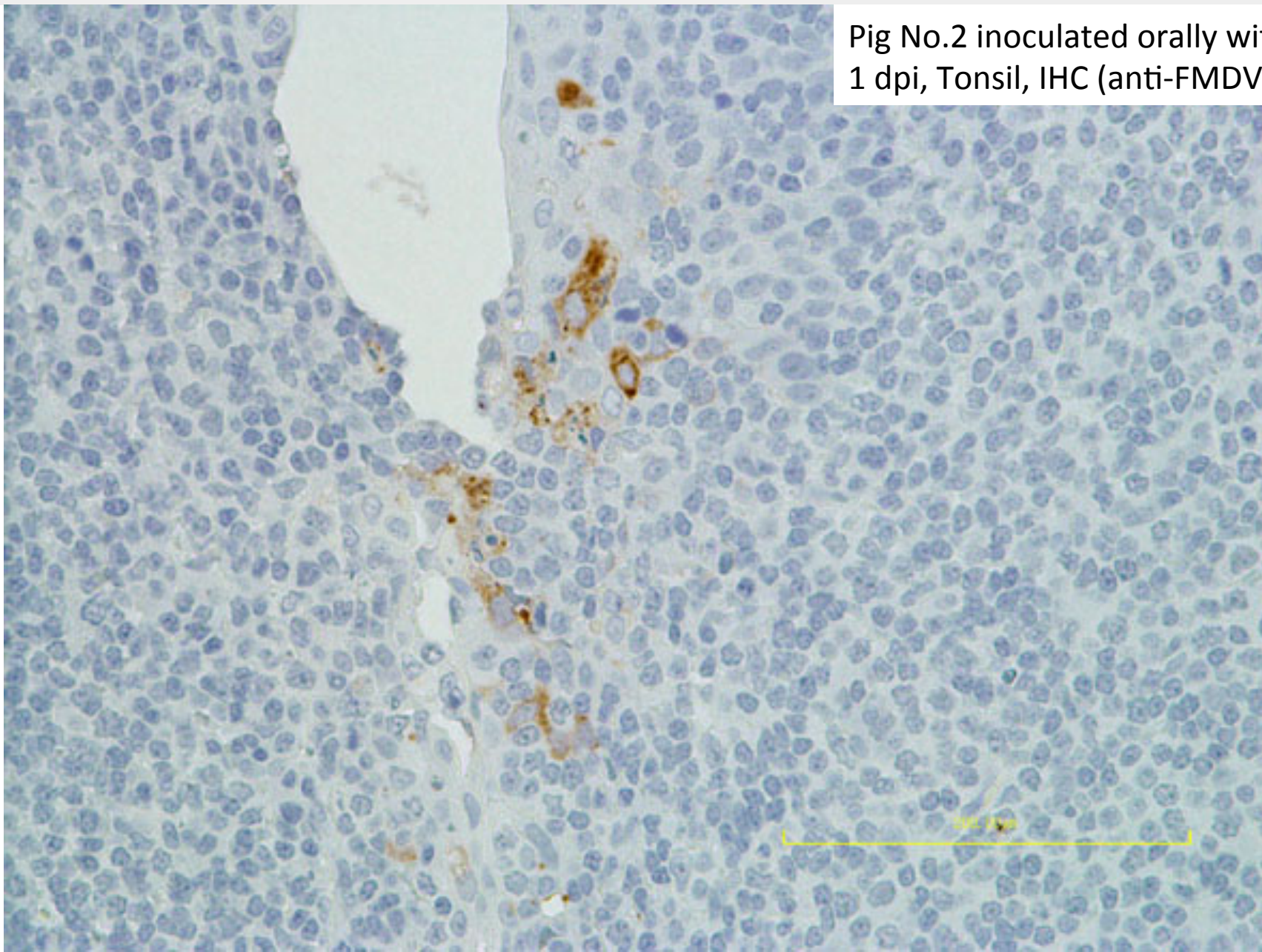
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Trachea	-/-	-/-	-/-	-/-	3.25/+	1.75/+
Lung	-/-	-/+	-/-	-/-	3.0/+	1.75/+
Esophagus	-/-	-/+	-/-	-/+	5.75/+	-/+
Stomach	-/-	-/-	-/-	-/+	2.5/+	-/-
Small intestine (ileum)	-/-	-/-	-/-	-/-	2.5/+	-/-
Large intestine (rectum)	-/-	-/-	-/-	-/-	2.5/+	-/-
Heart	-/-	-/-	-/-	-/-	3.0/+	-/+
Spleen	-/-	-/-	-/-	-/-	5.0/+	-/+
Thymus	-/-	-/-	-/-	-/-	3.0/+	1.75/+
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Parotid lymph node	-/-	-/-	-/-	-/-	6.0/+	-/+
Retropharyngeal lymph node	-/-	-/-	-/-	-/-	5.0/+	-/+
Cervical lymph node	-/-	-/-	-/-	-/-	7.5/+	-/+
Mandibular gland	-/-	-/-	-/-	-/-	3.0/+	2.5/+
Parotid gland	-/-	-/-	-/-	-/-	2.5/+	-/-
Skin of the nose	-/-	-/-	-/-	-/+	6.5/+	-/-
Skin of the coronet	-/-	-/-	-/-	-/+	8.5/+	4.5/+

The result of virus isolation and RT-PCR analysis from tissue samples

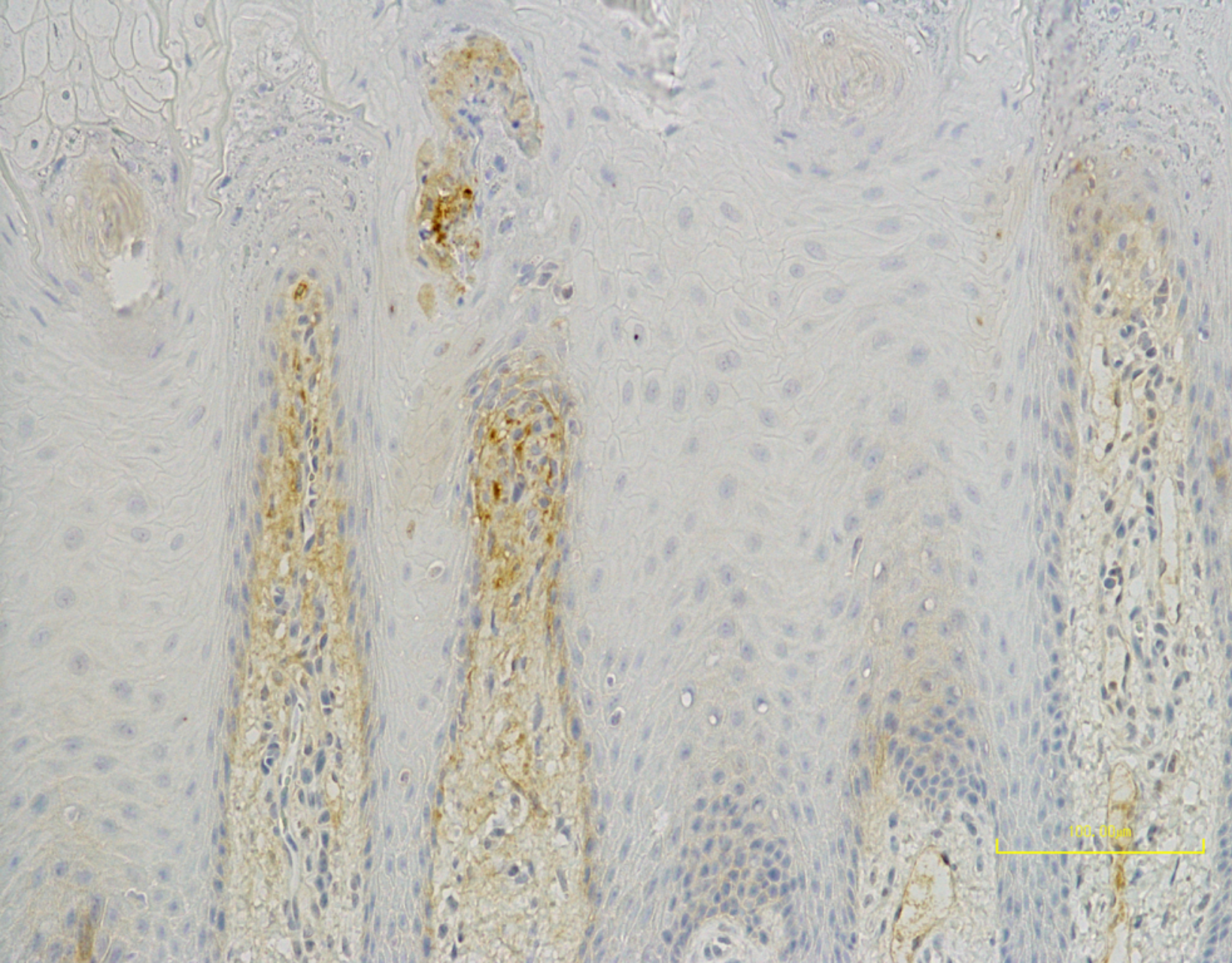
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Heart	-/-	-/-	-/-	-/-	3.0/+	-/+
Spleen	-/-	-/-	-/-	-/-	5.0/+	-/+
Thymus	-/-	-/-	-/-	-/-	3.0/+	1.75/+
Mandibular lymph node	-/-	-/-	-/-	-/-	6.25/+	-/+
Parotid lymph node	-/-	-/-	-/-	-/-	6.0/+	-/+
Retropharyngeal lymph node	-/-	-/-	-/-	-/-	5.0/+	-/+
Cervical lymph node	-/-	-/-	-/-	-/-	7.5/+	-/+
Mandibular gland	-/-	-/-	-/-	-/-	3.0/+	2.5/+
Parotid gland	-/-	-/-	-/-	-/-	2.5/+	-/-
Skin of the nose	-/-	-/-	-/-	-/+	6.5/+	-/-
Skin of the coronet	-/-	-/-	-/-	-/+	8.5/+	4.5/+

Pig No.2 inoculated orally with O/JPN/2010,
1 dpi, Tonsil, IHC (anti-FMDV type O)



Histological lesions and immunohistochemical viral antigen in the tongue.



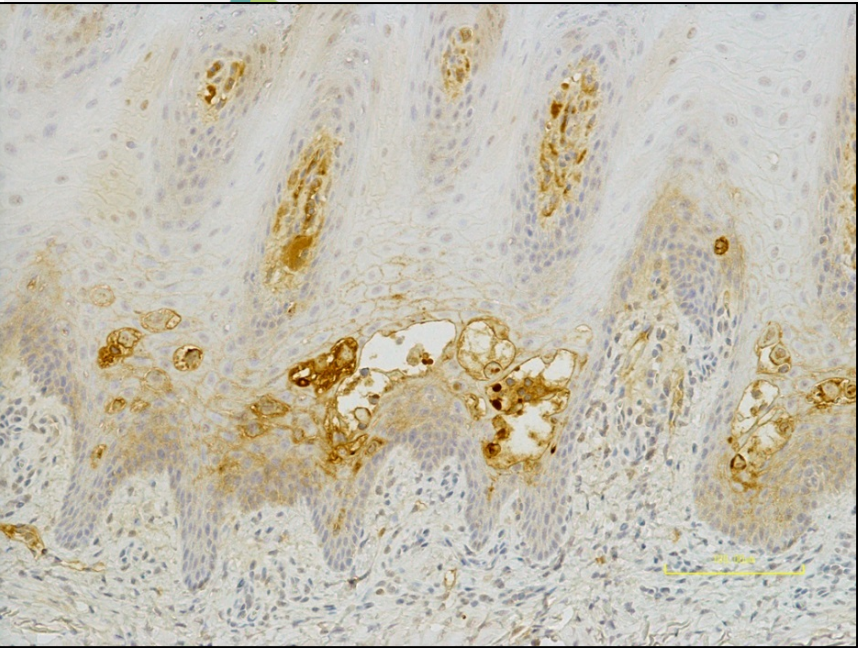


Pig No.2 inoculated orally with O/JPN/2010, 1 dpi, Epithelium of the tongue, IHC (anti-FMDV type O)

Viral antigens were detected at first in the pallia of connective tissue and perivascular prickle cell in the upper layer of the stratum spinosum in the tongue.

The direct epithelium invasion via the surface epithelial cells was not observed in this study.

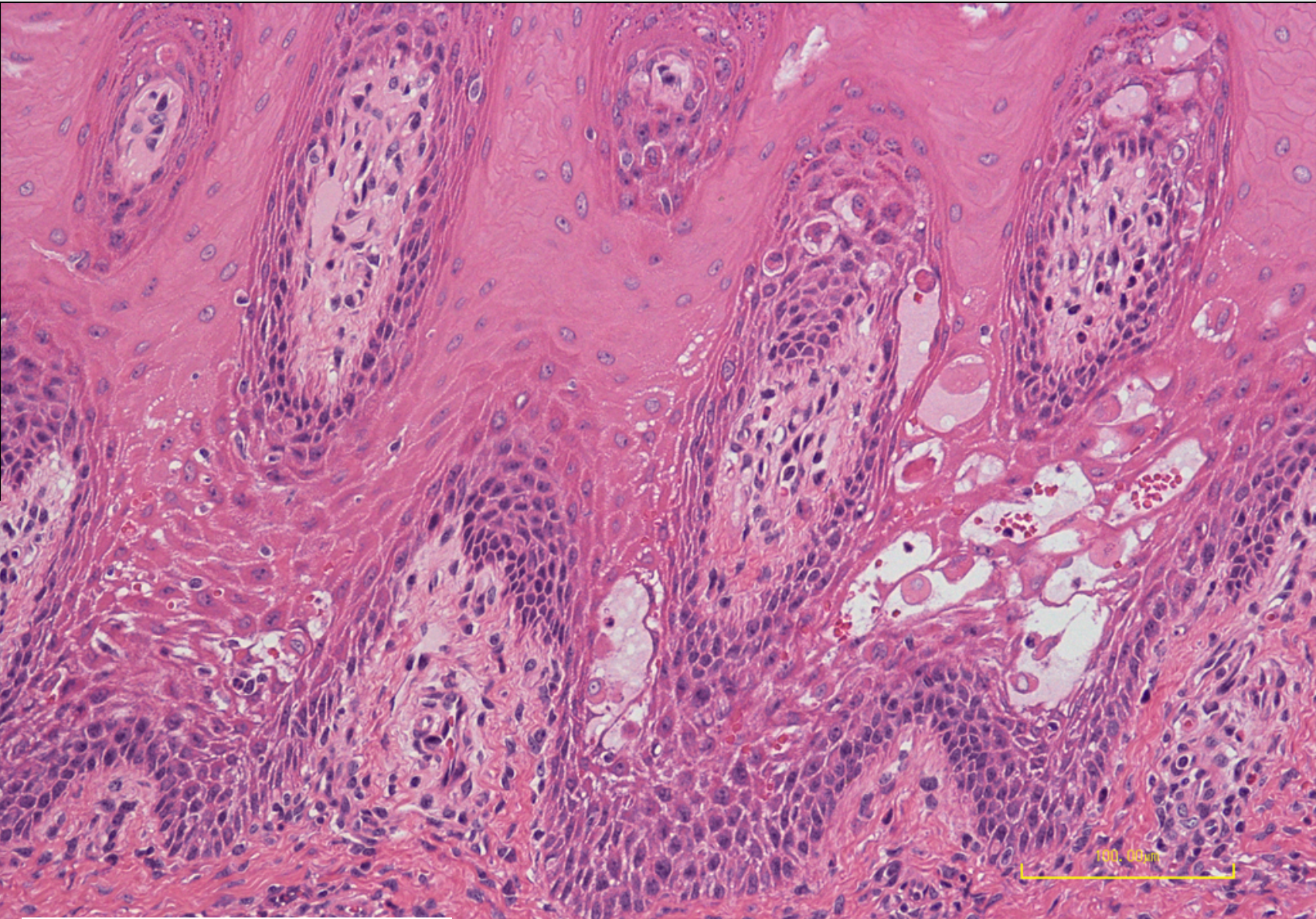
It is confirmed that the epithelium invation via the blood vessels is a major route of infection for FMDV.



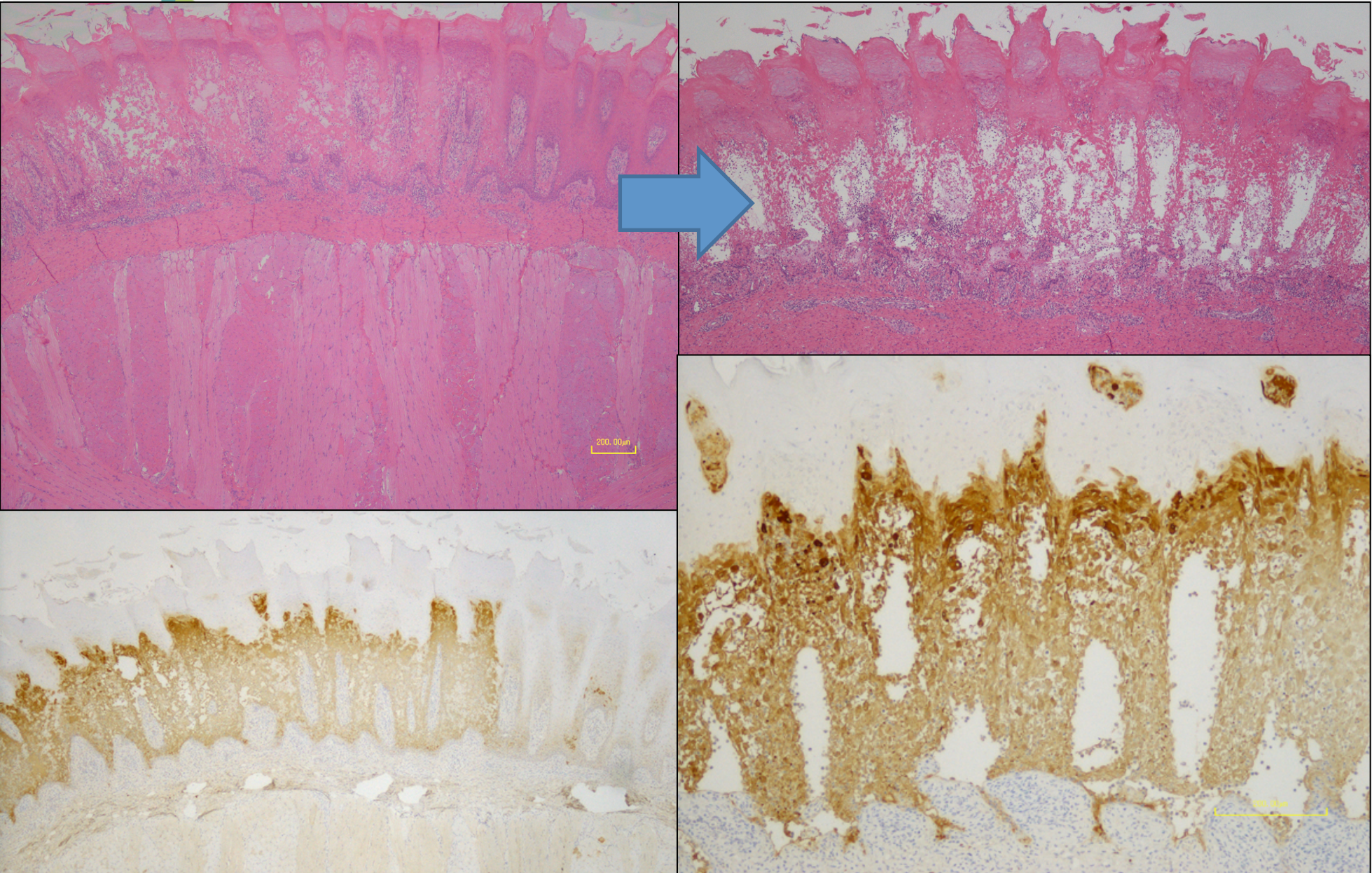
Pig No.4, 3 dpi, Tongue, IHC (anti-FMDV type O)

The epithelial lesion seemed to start on the single necrosis of the prickle cell in the middle to bottom layer of the stratum spinosum in the tongue. Those changes of single necrosis develop small vesicles.

The basal layer is intact and viral antigen is not detected in the basal layer.

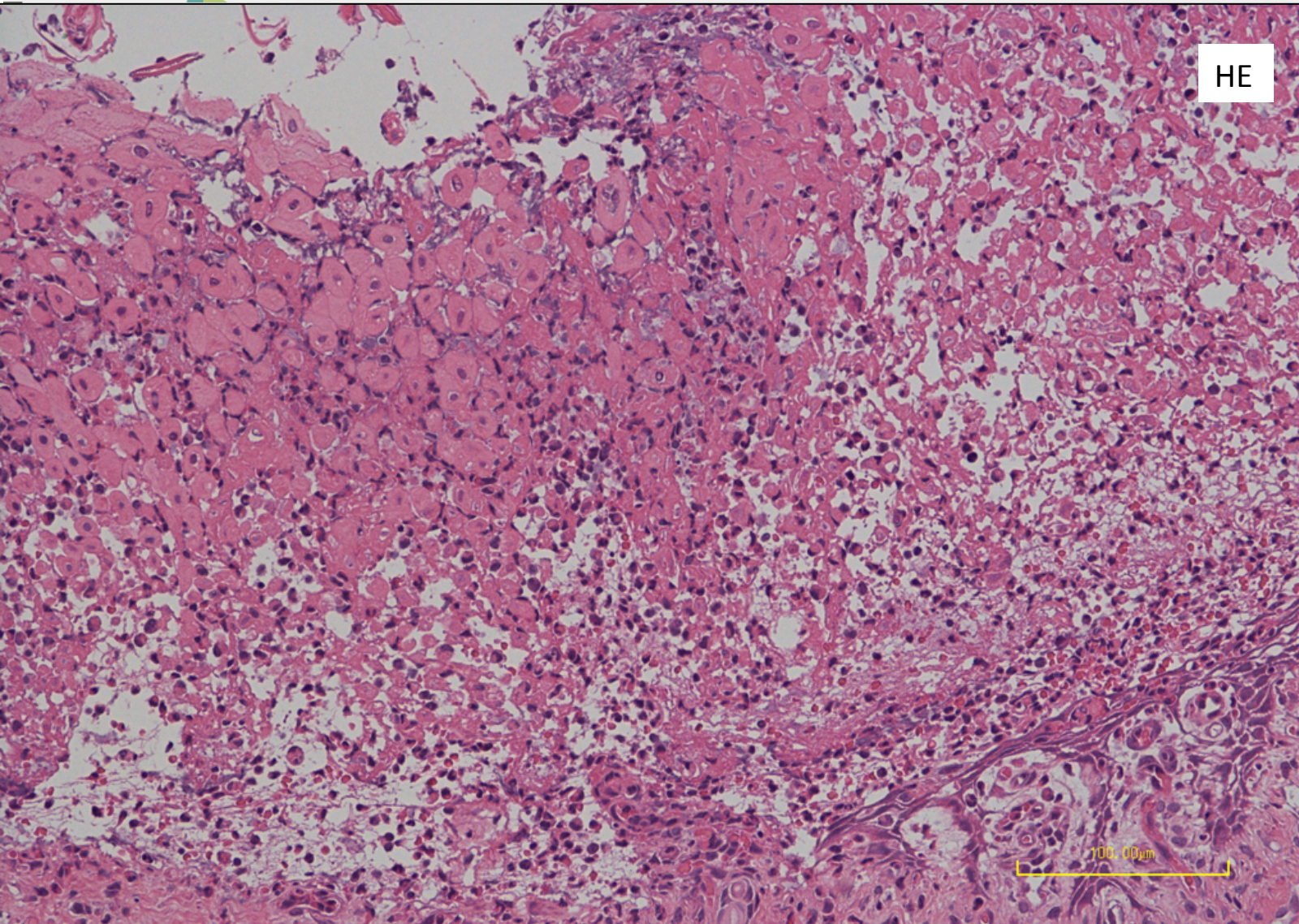


Pig No.4, 3 dpi, Tongue, HE

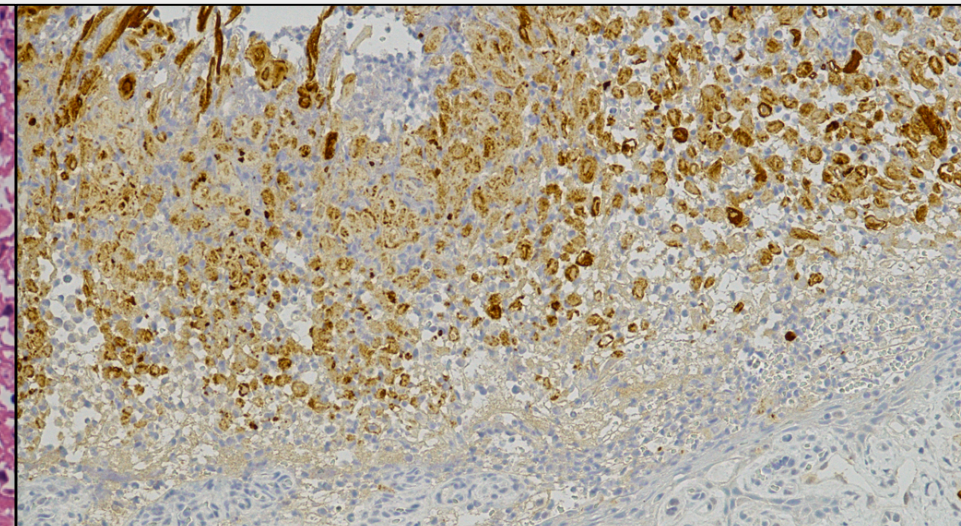


As necrotic lesions developed, small vesicles seemed to be fused together and develop large vesicles in the tongue.

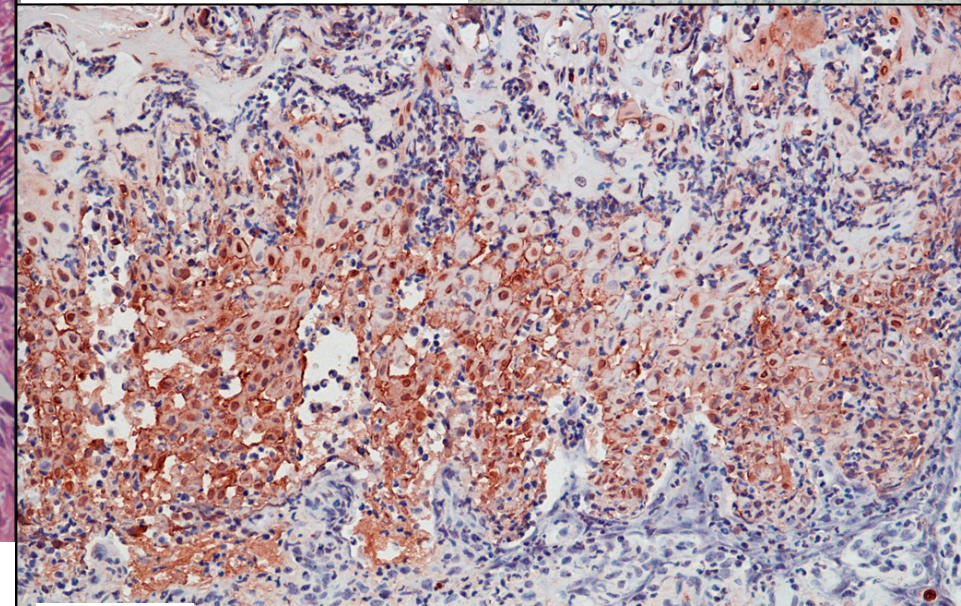
In this process, as pallia of connective tissue were destroyed and filling of the cavity with vesicular fluid, the vesicular lesion in the epithelium of the tongue developed as spongiform appearance.



HE

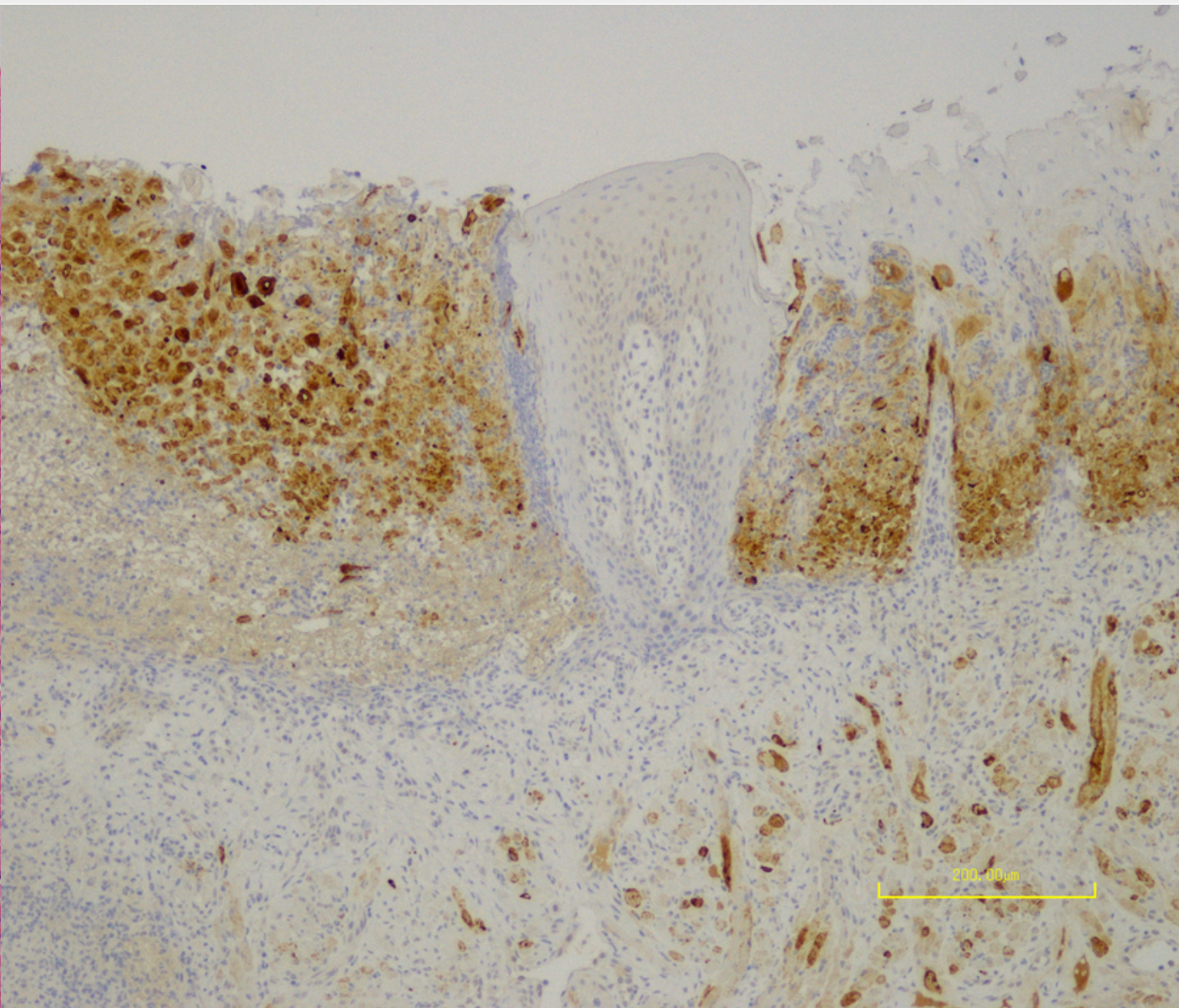
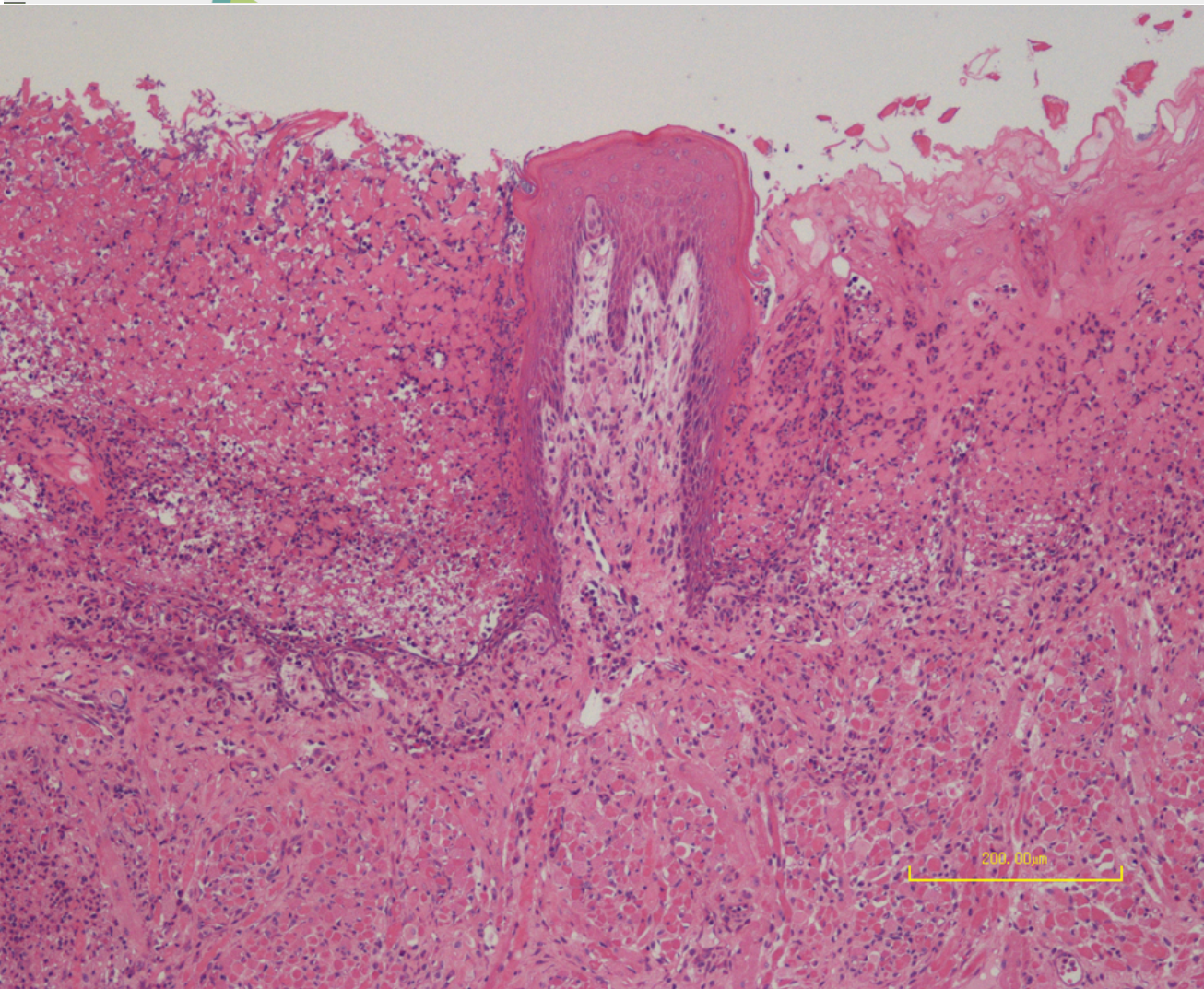


IHC (anti-FMDV type O)



TUNEL

The cell injury in the basal layer may be not by virus directly, but by the result of the lesion of surrounding tissue indirectly.



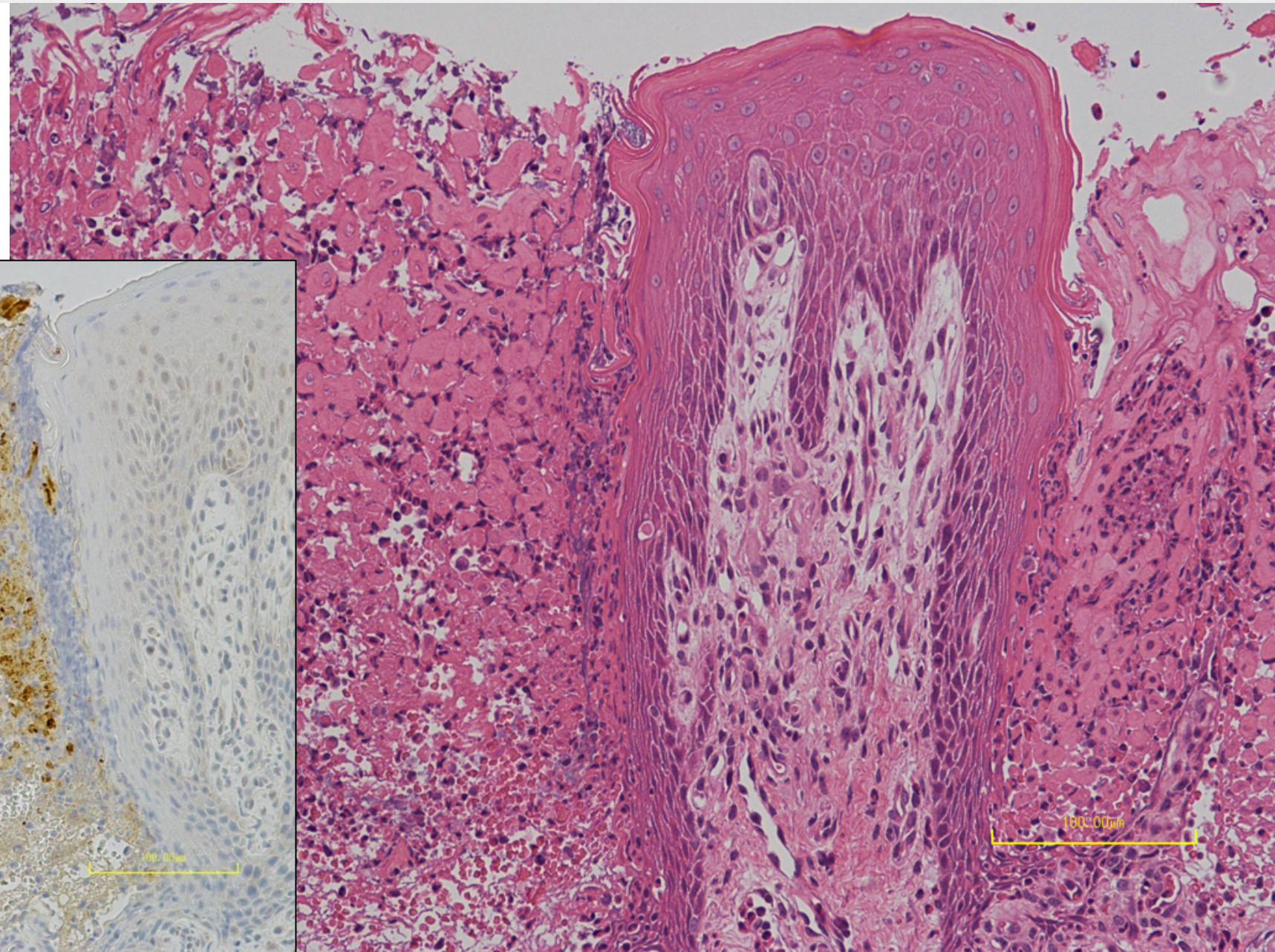
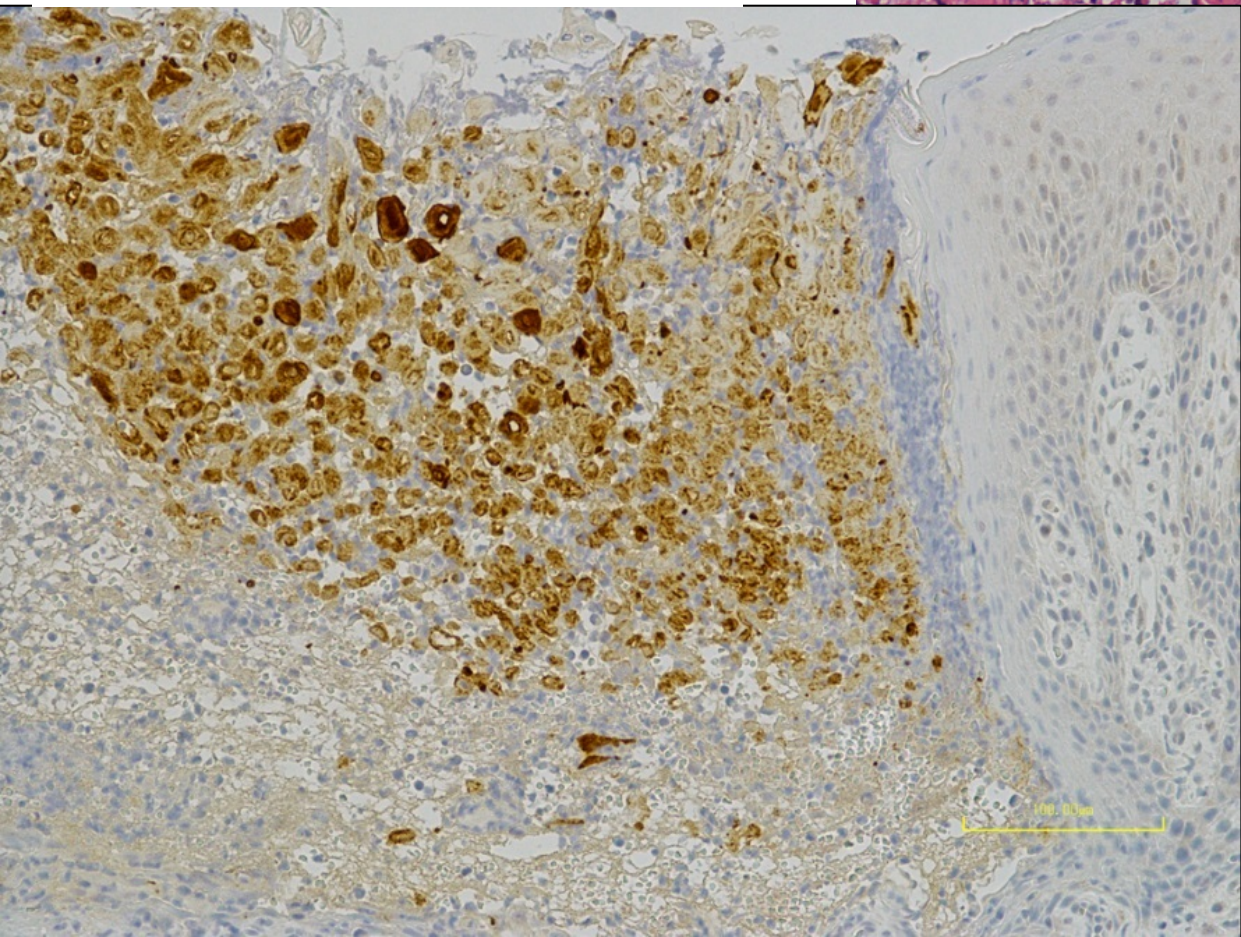
Pig No.4, 3 dpi, Tongue, HE

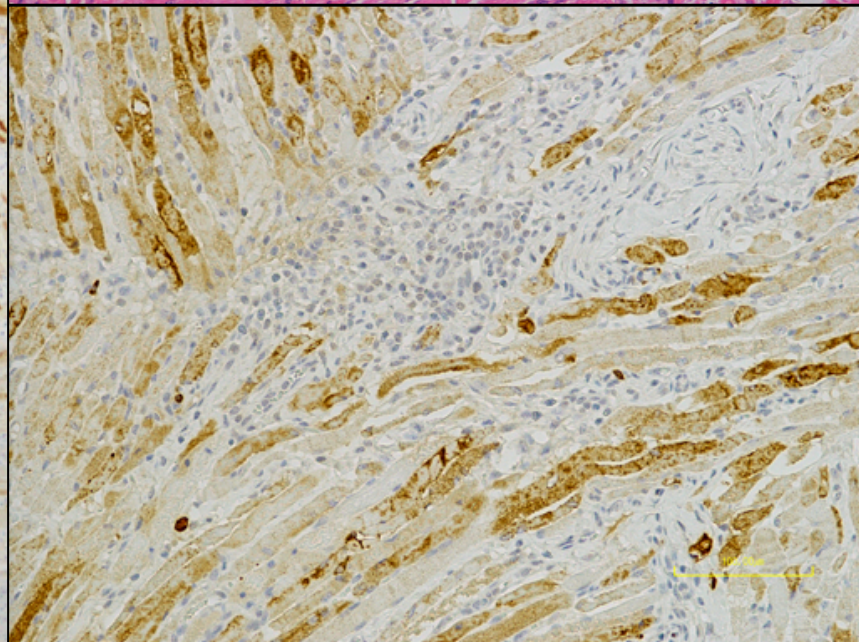
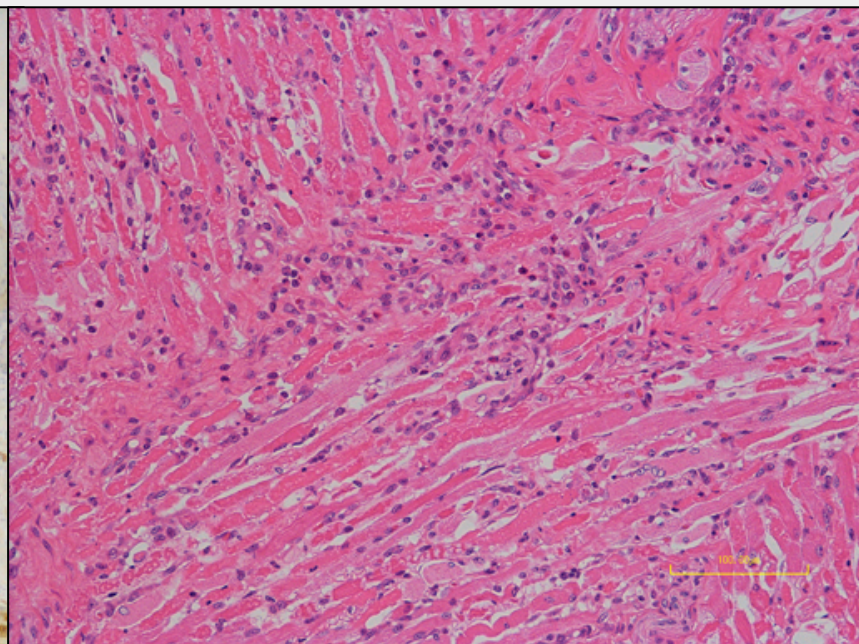
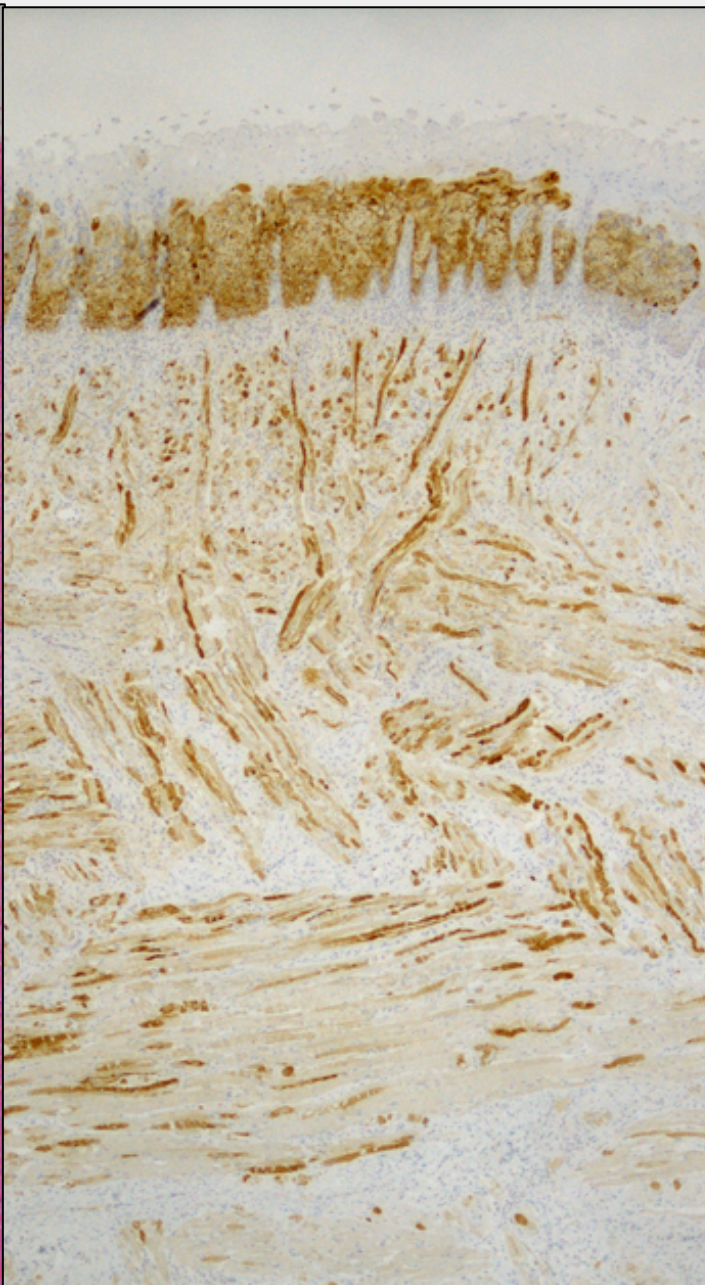
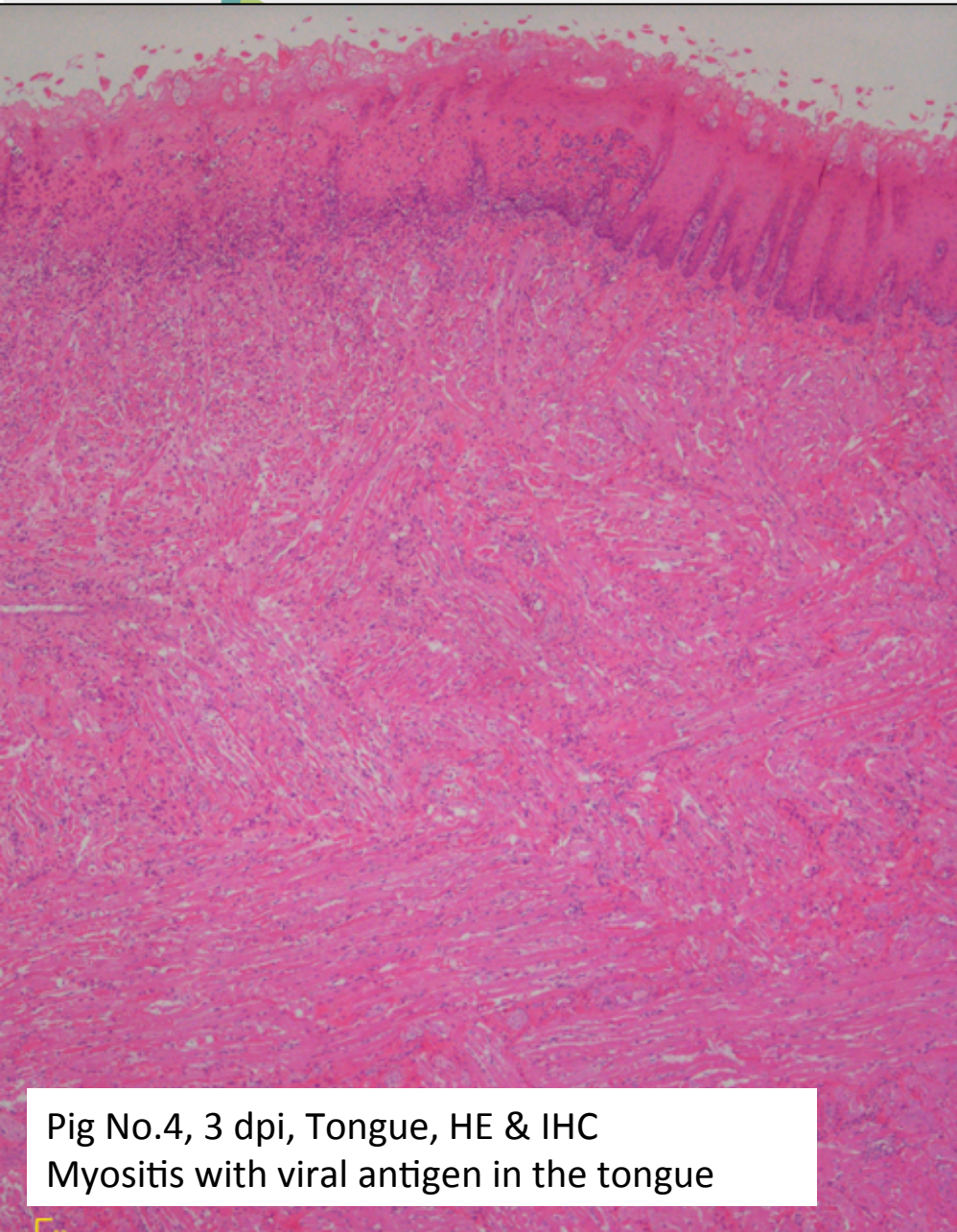
OS16

The papillae of the tongue was intact although the surrounding tissues were severely affected in this study.

Viral antigen is not detected in the papillae of the tongue.

Pig No. 4, 3 dpi, Tongue, HE & IHC

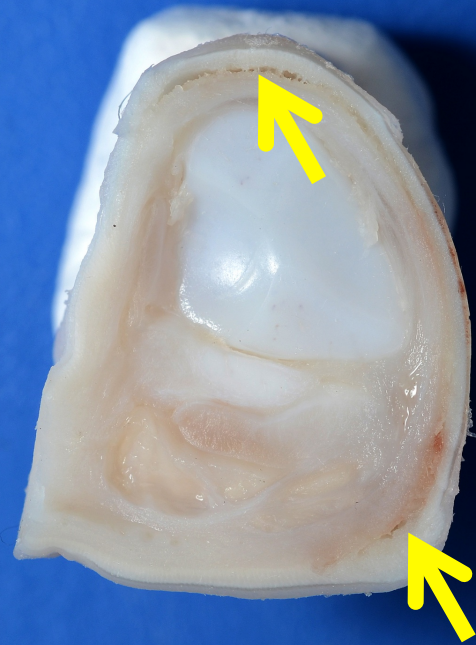




Pig No.4, 3 dpi, Tongue, HE & IHC
Myositis with viral antigen in the tongue

Histological lesions and immunohistochemical viral antigen in the foot.

Grossly, only blanch appearance, Vesicular lesions were detected at the cut surface.



1 dpi

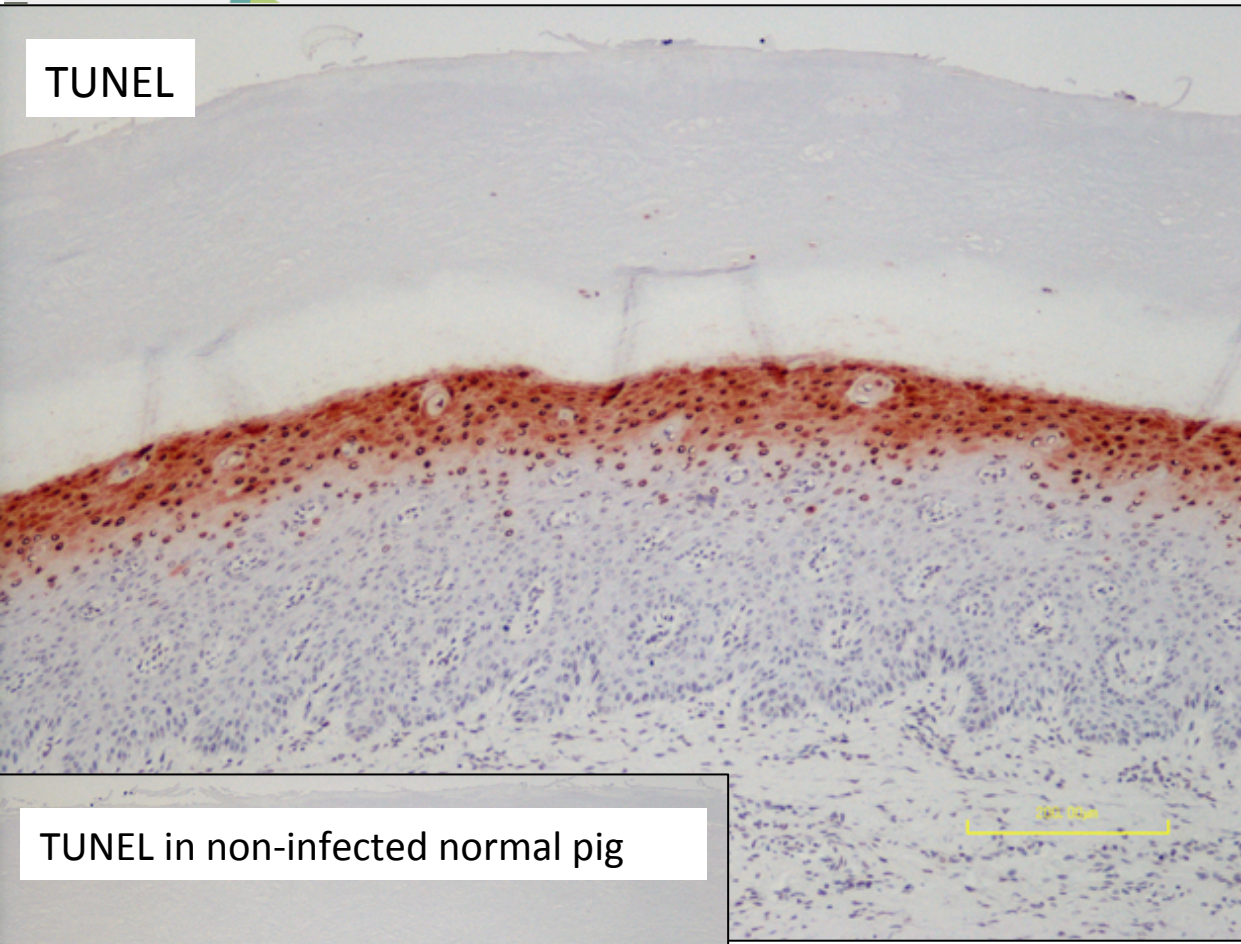
Grossly, large vesicle was seen in the interdigital to bulb of the heel, Vesicular lesions at the cut surface.



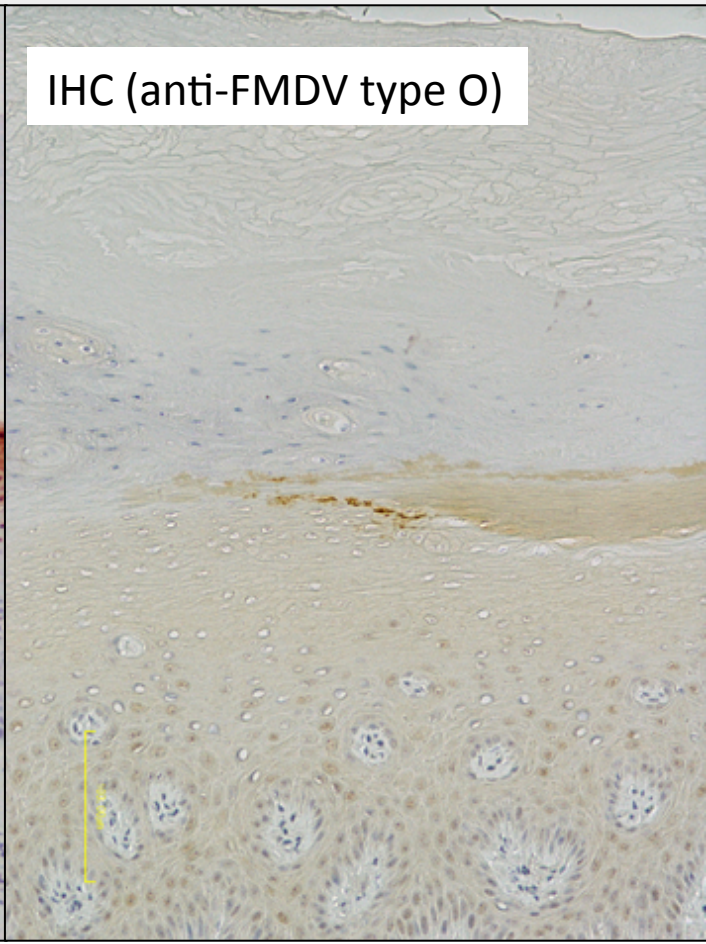
3 dpi

Vesicular lesions in the coronet were appeared at the side or ridge (arrows) at first in this study, then developed all side of the coronet.

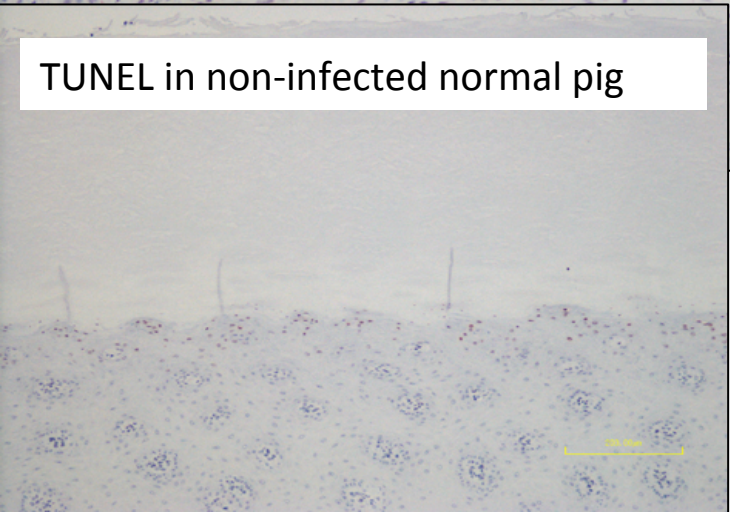
TUNEL



IHC (anti-FMDV type O)

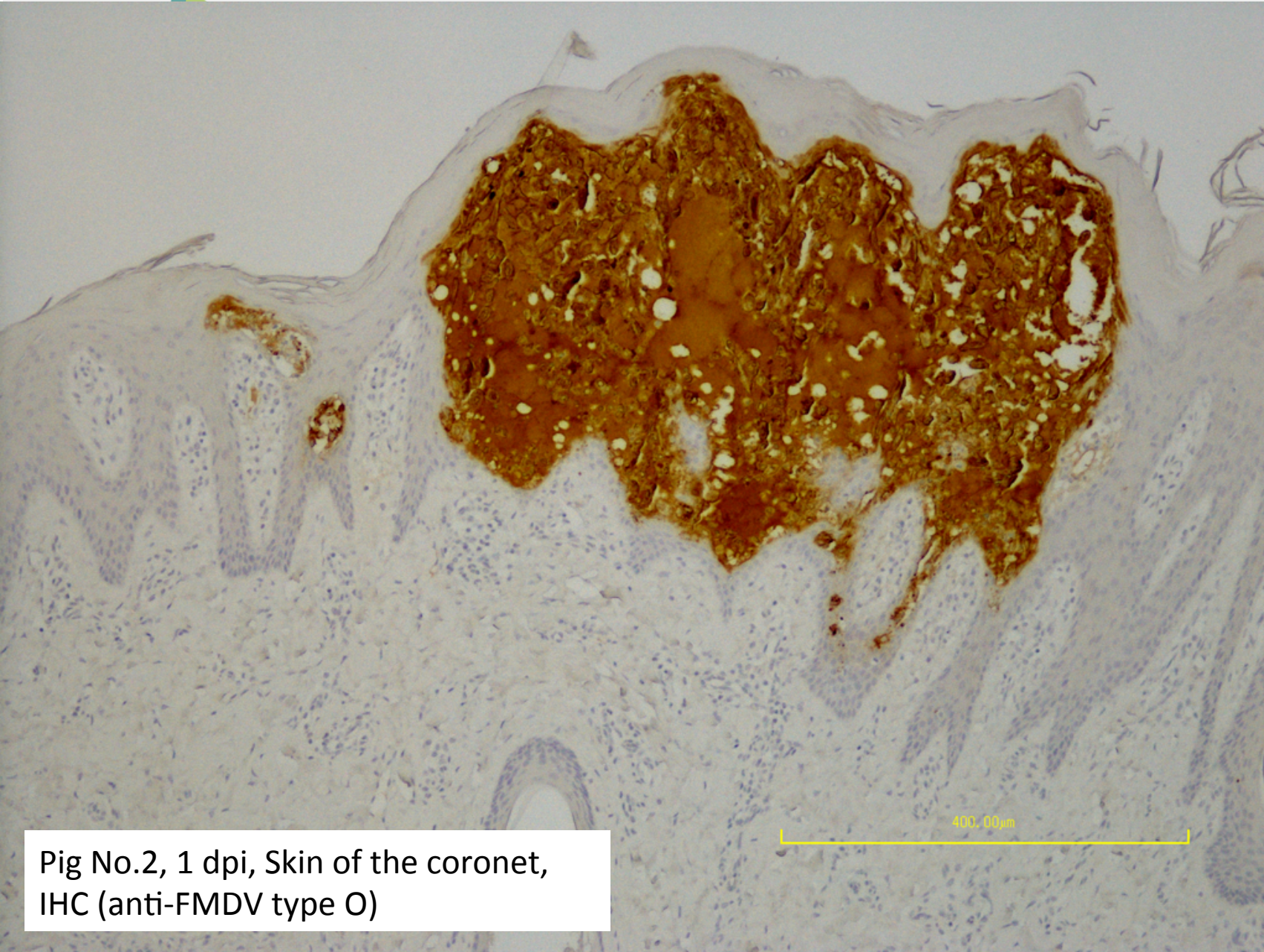


TUNEL in non-infected normal pig



Pig No.3, 1 dpi,
Skin of the coronet,
TUNEL & IHC
(anti-FMDV type O)

Before cellular injury appeared, TUNEL positive reaction has already seen prominently in lamellar at the upper layer of the stratum spinosum in comparison with those in non-infected normal pig. In this stage, viral antigen is not prominent, only seen faintly at the surface of the stratum spinosum.



The lesion in the epidermis of the coronet and heel seems to start as focal necrosis in the upper to middle layer of the stratum spinosum.

Those focal necrosis may develop rapidly to massive necrosis with prominent viral antigen.

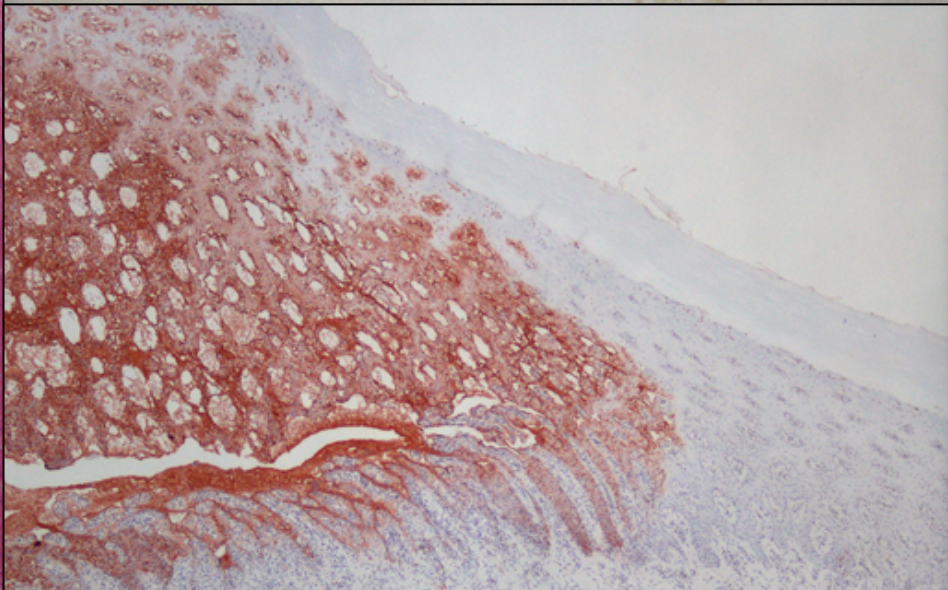
Pig No.2, 1 dpi, Skin of the coronet, IHC (anti-FMDV type O)



Then, cleft (arrow) was appeared
in the center of necrotic lesion.

Pig No.2, 1 dpi, Skin of the coronet,
IHC (anti-FMDV type O)

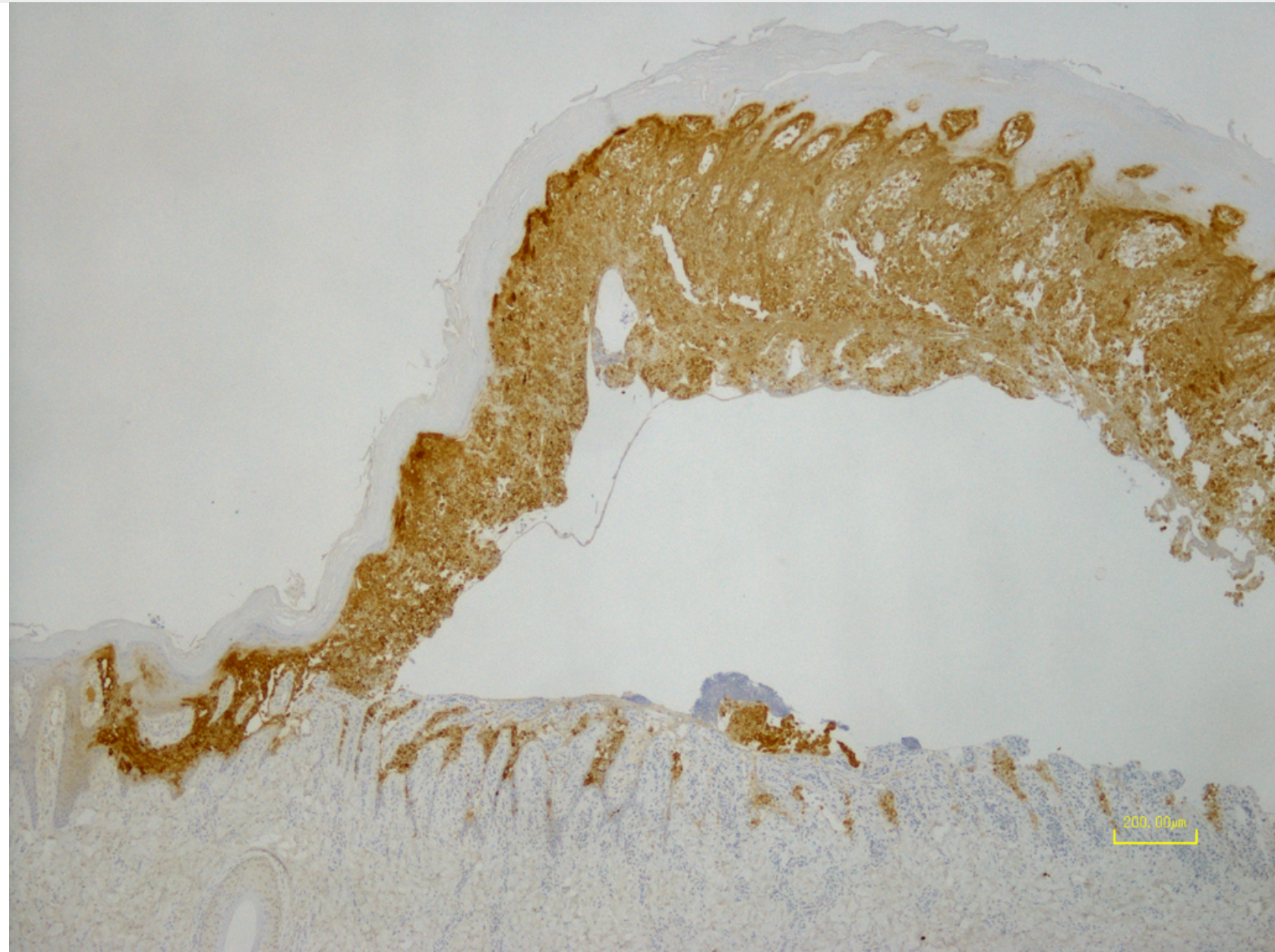
Pig No.2, 1 dpi, Skin of the coronet,
HE, IHC (anti-FMDV type O) &TUNEL



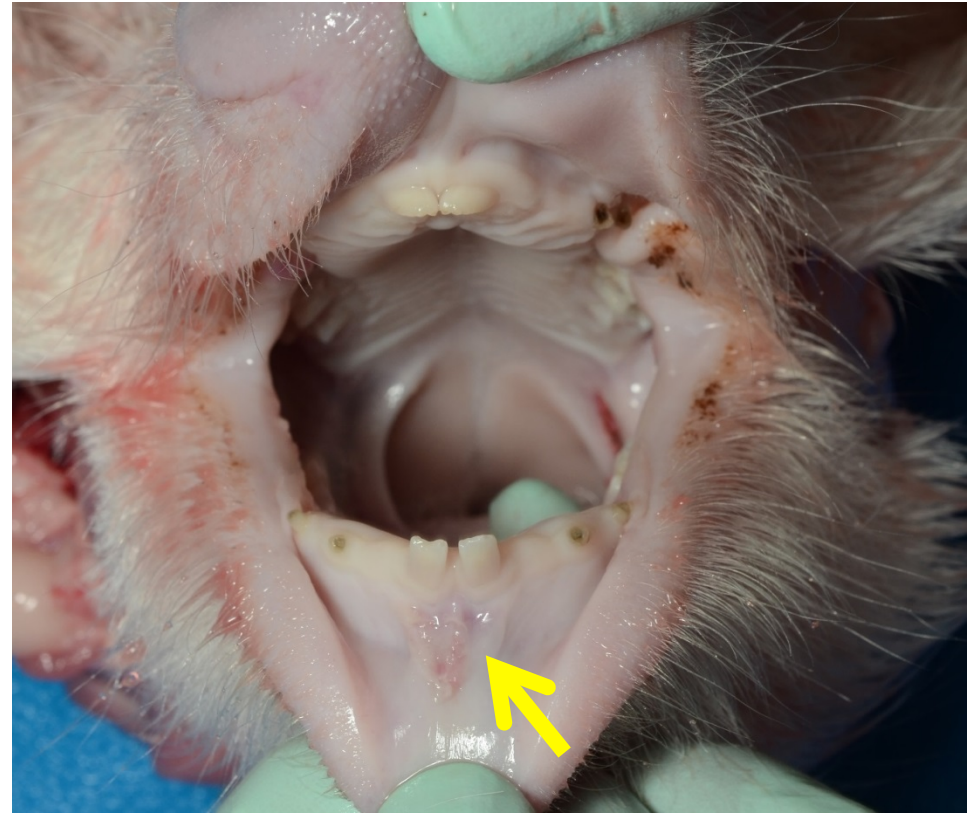
The epidermal lesion with those cleft showed viral antigen positive and TUNEL-positive prominently and developed further into vesicles by separation of the epidermis from the underlying tissue.

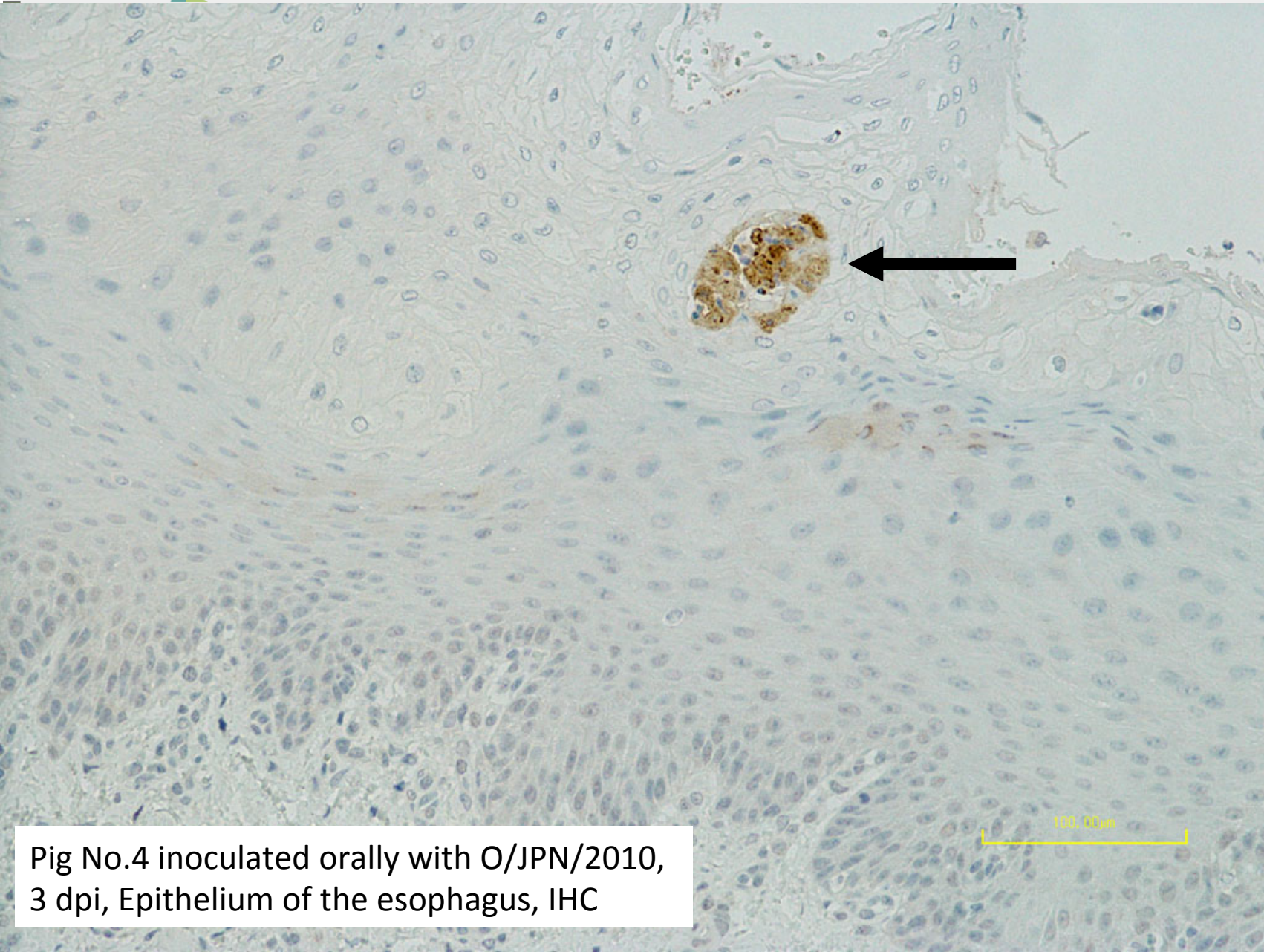
Pig No.4, 3 dpi, Skin of the coronet,
IHC (anti-FMDV type O)

Finally the epidermal lesion develop further into vesicles by filling of the cavity with vesicular fluid.



Histological lesions and immunohistochemical viral antigen in other organs examined.

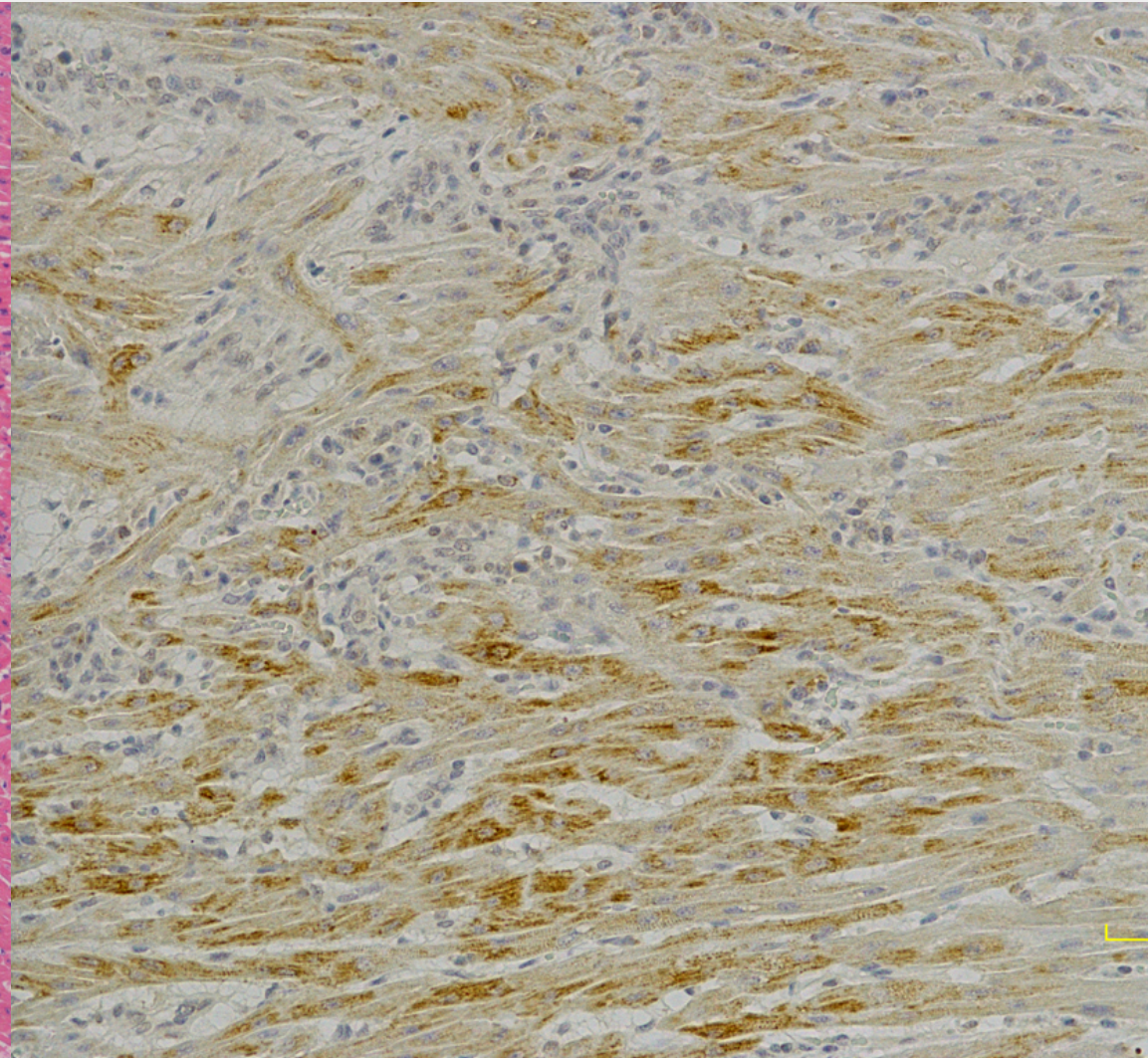
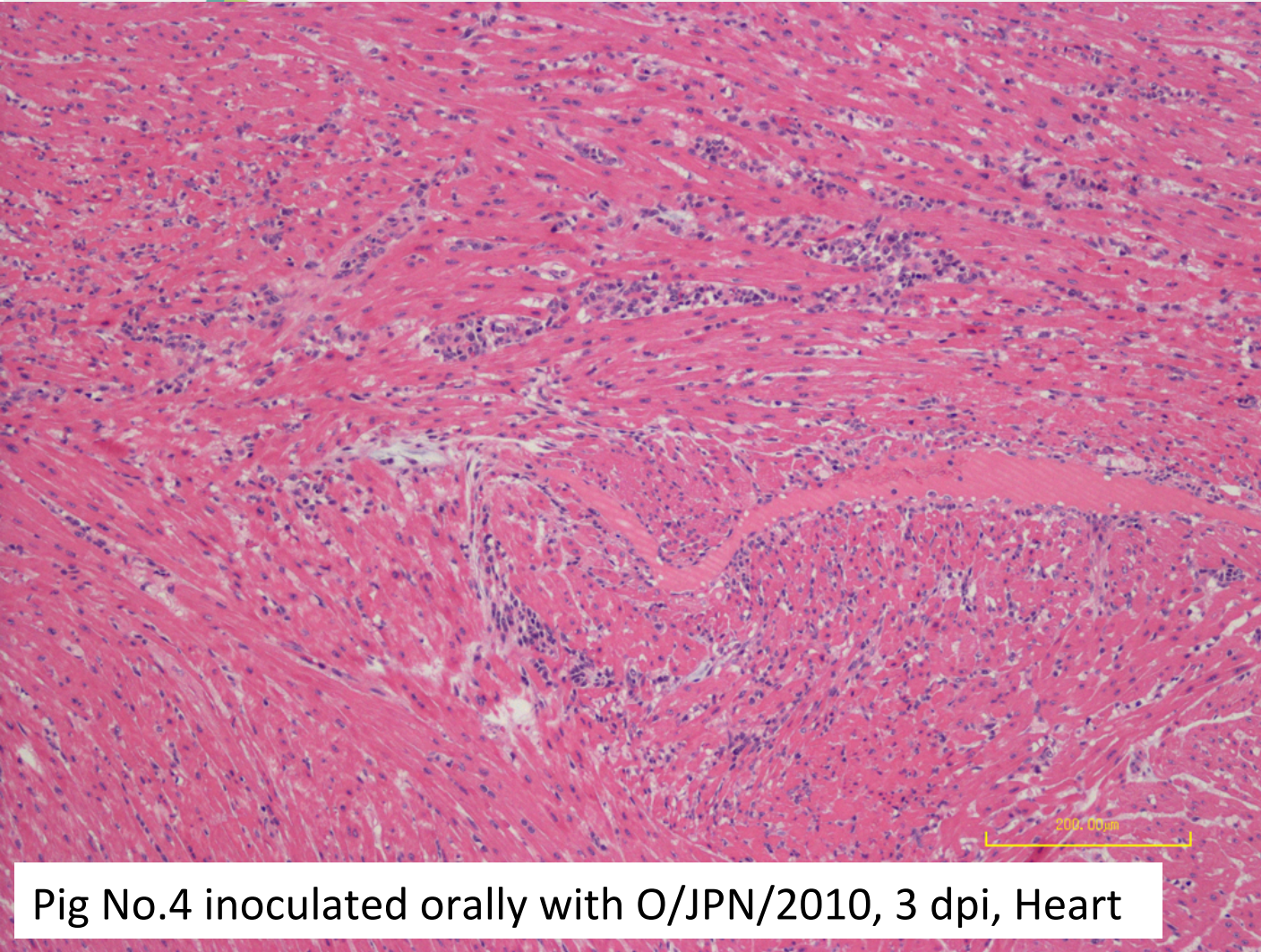




In the esophagus, multifocal necrosis with viral antigen are observed in the upper layer of the epithelium.

The basal layer is intact and viral antigen is not detected in the basal layer in this study.

Pig No.4 inoculated orally with O/JPN/2010, 3 dpi, Epithelium of the esophagus, IHC



Pig No.4 inoculated orally with O/JPN/2010, 3 dpi, Heart

In this study, obviously visible lesion in the heart was not observed in any pig at necropsy. However, myocarditis with prominent viral antigen was histologically appeared in all three pigs on 3 dpi.

Conclusion

It was confirmed that Characteristic lesions that commonly seen in pigs of FMD were observed in pigs inoculated with O/JPN/2010 by natural infectious routes.

The distribution of the lesion and virus and pathological changes in acute stage of infection in this study were almost same as those in previous reports.

It is confirmed that inoculated FMDV prefer to infect with prickle cell in the stratum spinosum in the tongue and skin of the coronet and heel and lead infected cells to cell death by apoptosis in this study.

On the other hand, viral antigen is not detected in the basal layer of the epithelium and the papillae of the tongue.

In this study, it was appeared that pathological processes of the development of vesicular lesion in the epidermis of the coronet and heel were different from those in the epithelium of the tongue in pigs inoculated with FMDV O/JPN/2010 isolate.

Thanks to ...

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